DIPLOIDS FROM UNFERTILIZED EGGS IN HABROBRACON

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FEMALES

The occurrence of impaternate females (females from unfertilized eggs) in the parasitic wasp *Habrobracon juglandis* (Ashmead) has previously been reported (Speicher, 1934). Such females occur sporadically from various virgins, and regularly constitute about 1 per cent of the F_2 population from F_1 virgins produced by outcrossing females from tapering or reverted tapering stocks. The hypothesis was offered that these diploid impaternate females might be produced by the failure of the second maturation division in the unfertilized egg and, carrying two chromatids from a single tetrad, would provide material for studying the mechanics of crossing-over.

When F_1 virgins were heterozygous for recessive factors, F_2 impaternate females occurred in the ratio of one homozygous dominant, to two heterozygotes, to one homozygous recessive for each locus involved. This 1 : 2 : 1 ratio was at variance with results obtained in other organisms where more than one strand may be recovered from a single tetrad, notably *Drosophila* (Anderson, 1925) and *Neurospora* (Lindegren, 1933). Here the first maturation division is reductional at the spindle fiber and in dyads from that division the homozygosis of any locus depends upon the amount of crossing-over between it and the fiber, and hence is a function of its location along the chromosome. In *Habrobracon*, except for the locus of the sex-linked factor fused (Whiting and Speicher, 1935), the amount of homozygosis was consistently 50 per cent for all loci tested, even including those of two recessives known to be linked and separated by a distance of ten units.

It was realized from the beginning of the work that other hypotheses could be advanced to explain the formation of impaternate females. And it was apparent that the 1 : 2 : 1 ratio obtained would be expected for all loci if the two homologous chromosome strands of an impaternate female came from two tetrads, independent and com-

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pletely reduced, rather than from a single tetrad which underwent only partial reduction. As an investigation of oögenesis in *Habrobracon* had already been begun (Speicher, 1936), it seemed best to suspend further genetic studies until the method of formation of impaternate females could be determined cytologically. Those findings are here reported for the first time.

Since impaternate females had been shown to be genetically diploid, whereas their sibs are haploid, a cytological analysis of their formation seemed practical. According to past work on other forms at least three basic hypotheses could be considered. First, one of the two maturation divisions of an unfertilized egg could be suppressed, as was observed by Silvestri (1908) in the wasp *Prospalta*. This would leave a cleavage nucleus containing the diploid number of chromatids, which would restore the diploid number of chromosomes if the homologues separated. Second, fusion of two reduced egg nuclei present in a binucleate egg also would restore the diploid condition. Third, the egg might originate as a tetraploid, undergo reduction and thus become diploid. A fourth hypothesis, the doubling of chromosomes in the haploid egg during cleavage, is eliminated because tests have shown that impaternate females may be genetically heterozygous.

TABLE I

Progeny from No. 25/reta virgin females.

F₁ virgin ♀ ♀	1	2*	3	4	5	6	7*	8	9	10	11
Sons	83	0	91	26	95	97	5	67	29	113	63
Daughters	1	2	0	0	5	0	0	0	0	0	7
Eggs collected	49	3	50	36	46	61	11	29	48	56	51

* Died before completion of experiment.

The first two theories were tested together. Over 300 eggs were collected from virgin females produced by crossing reverted tapering and stock 25. They were fixed at first cleavage prophase and stained by the Feulgen whole-mount method. The same females produced collectively 15 impaternate daughters among 724 sons, over 2 per cent of the total. Among the eggs studied approximately the same proportion would be expected to show cytological differences if either theory were correct. Suppression of a maturation division would result in a decrease in the normal number of polar nuclei formed at the egg margin. A binucleate egg would be expected to show two groups of polar nuclei, totaling twice the normal number. An examination of all eggs revealed none showing either of these two differences.

Treatment of the third hypothesis requires chromosome counts during maturation; accordingly it was necessary to obtain a new lot of eggs fixed at an earlier stage than the above. It had been noticed previously that some virgin females produced impaternate daughters in small groups while others in the same experiment produced no daughters whatever. Inclusion in the data of the offspring from the latter virgins lowers the percentage of impaternate females among total offspring. It was therefore possible to raise the expected percentage of exceptional eggs by selecting eggs only from virgins known to be producing impaternate daughters. This was accomplished as follows. Eleven females from a cross of reverted tapering by stock 25 were placed with host caterpillars. Eggs laid upon the caterpillars during seven consecutive days were fixed at first anaphase and temporarily stored in alcohol. Eggs laid at night over the same period were allowed to develop, in order to indicate which of the eleven females were thelytokous. Results are shown in Table I.

Fig. 1.

Fig. 2.

Fig. 1. First anaphase of normal egg. × 3,000.
Fig. 2. First anaphase of tetraploid egg. × 3,000.

Seven females, producing a total of 428 sons, had no daughters, while the remaining four produced 241 sons and 15 daughters. The 149 eggs collected from the latter four females were then stained by the Feulgen whole-mount technique. One hundred and ten of these were in condition to study; the remainder were either lost in handling or were collapsed. Ninety-eight eggs were unquestionably diploid, seven were unquestionably tetraploid and five more were questionably tetraploid. The clear cases of tetraploidy showed twenty chromosomes, presumably bivalents although individual chromatids have never been observed in *Habrobracon* oögenesis due probably to their small size, moving to one or to each pole. Normal diploid eggs show only ten chromosomes going to each pole, Figs. 1 and 2.

These cytological data, and the fact that impaternate females come in groups from certain mothers suggest the probability that production of tetraploid eggs, as developed from patches of tetraploid ovarian tissue, is responsible for the appearance of diploid impaternate females among haploid brothers.

MALES

Diploid males of biparental origin have been reported repeatedly in *Habrobracon*. Since diploid females are produced by virgins as a result of tetraploidy in egg cells, it seems plausible that diploid impaternate males may be produced in the same way. According to Whiting's scheme of sex-determination in *Habrobracon* (Whiting, 1933) those eggs which were diploid after reduction and contained sex chromosomes X and Y would produce impaternate females, while those which contained chromosomes XX or YY would produce diploid impaternate males. If distribution of chromatids is random the number of diploid females and males produced should be equal.

TABLE II

Data from tests for diploid impaternate males.

<table>
<tr><th rowspan="2">F₁ ♀♀</th><th rowspan="2">F₂ haploid ♂♂, A or a</th><th colspan="3">F₂ impaternate ♀♀</th></tr>
<tr><th>AA</th><th>Aa</th><th>aa</th></tr>
<tr><td>o^{1}/o^{d}</td><td>6222</td><td>6</td><td>10</td><td>5</td></tr>
<tr><td>*Le/le*</td><td>6070</td><td colspan="2">17</td><td>6</td></tr>
<tr><td>*St/st*</td><td>4408</td><td>4</td><td>2</td><td>0</td></tr>
</table>

Three experiments were set up in an effort to produce diploid impaternate males that could be distinguished genetically from their haploid brothers. Since the highest percentages of impaternate F_2 diploids had previously resulted from outcrosses of tapering and reverted tapering females, tapering was again used as the maternal stock in one experiment. In the other two it was necessary to introduce recessive factors into the cross through the females. In order to insure the occurrence of thelytoky in these cases the recessives were repeatedly bred up to reverted tapering, and a stock related to it but having the desired genetic constitution was then derived and supplied the females for the parental crosses. In each experiment F_1 virgins were produced carrying both of two allels that give rise to a distinct phenotype when they are heterozygous.

Results are given in Table II where the first column shows the pairs of allels used to make the heterozygotes recognizable phenotypically, the second column gives the number of normal haploid males in the F_2, and the third column gives the classes of impaternate females. Theoretically diploid impaternate males should have occurred in the same numbers and in the same genetic ratio (1 homozygous dominant, AA : 2 heterozygotes, Aa : 1 homozygous recessive, aa) as did the impaternate females, although among the males only the heterozygotes (Aa) would have been distinguishable from their normal haploid brothers.

No diploid males were found and the experiments are published at this time because the stocks with which they might be repeated are no longer extant and the work is finished unless or until the thelytokous tendency reappears in *Habrobracon*. However, results are not considered entirely convincing, nor is the evidence against the occurrence of diploid impaternate males considered conclusive. The following paragraphs tell for each experiment why the tests were not as satisfactory as had been expected.

o^i/o^d.—Wasps heterozygous for the allelomorphic eye colors ivory (o^i) and dahlia (o^d) had previously been distinguished phenotypically by a lighter color than the dark red of homozygous dahlia (Torvik, 1931). In this experiment light dahlia was recognizable in the F_2 females, where it was checked by breeding tests. In the F_2 males, however, the color varied widely, grading from dark to light. Forty males were recorded as having eyes as light as their ten heterozygous sisters.

F_1 virgins in the eye color experiment were also heterozygous for the sex-linked factor fused (*fu*, antennæ and tarsi). Diploid impaternate daughters would be heterozygous for the sex region (XY), and also for fused except where cross-overs had occurred betewen fused and the sex region (10 per cent of the cases). Of the 21 impaternate females produced none was homozygous fused, and wherever it was possible to make breeding tests fused males occurred among their progeny, so that actually no cross-over cases were found. Diploid impaternate males, on the other hand, would be either XX or YY, and homozygous for *fu* or *Fu* except where cross-overs had occurred. Thus almost half the diploid males would carry two fused genes. C. H. Bostian had previously noted that the presence of two fused genes in diploid biparental males frequently made the antennæ shorter than those of fused haploid males where only one fused gene occurs. It was hoped that here extreme shortness of antennæ might serve as an additional tag for diploidy. However, antennal length proved to be too variable to be of any service, grading gradually from long to short.

Le/le.—In heterozygotes for the gene lemon (*le*, body color) pigmentation is normal except for the basal segments of the antennæ which are yellow instead of dark brown. The dominant factor Minnesota yellow (*My*, base of antennæ) produces exactly the same phenotype at high temperatures. It is homozygous in reverted tapering stock, and an effort was made to breed it out before this experiment was begun, but its presence was simply hidden by temperature changes. In the F_2 both *My* and *le* segregated. Of the impaternate females with normal body color it was impossible to tell which carried lemon. All but one were recorded as having yellow antennal bases due to the presence of one or the other or both of the mutant genes. If diploid males heterozygous for lemon were present, it was impossible to distinguish them from their haploid Minnesota yellow brothers.

St/st.—The experiment using the stumpy gene (*st*, legs) was abandoned because the number of F_2 impaternate females was so small that the expectancy for heterozygous diploid males fell so low as to make the search unprofitable, in spite of the fact that this was the one case where parental females had been of pure tapering stock.

SUMMARY

1. Cytological evidence shows that unfertilized eggs which give rise to females are tetraploid before reduction and therefore diploid after reduction.

2. No diploid males from unfertilized eggs were found among 16,700 males examined.

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